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14. ABSTRACT
Akt is an important kinase in prostate cancer, being activated downstream of growth factor receptors and downstream of PTEN mutations. Akt activity is controlled through two mechanisms, phosphorylation by Pdk1 and by Pdk2. Pdk2 is the mTORC2 complex, which controls phosphorylation of Akt at Ser473. We have recently shown that IKKα, a component of the IKK complex which controls NF-κB activation, regulates mTORC1 downstream of Akt activation. Here we explore a role for IKKα in controlling mTORC2 activity. Our data indicate that IKKα associates with mTORC2 in prostate cancer cells and regulates its activity towards Akt at Ser473. Knockdown of IKKα blocks Akt activity in prostate cancer cells. We propose to determine the mechanisms whereby IKKα controls mTORC2 activity and to determine if loss of IKKα in a animal model for prostate cancer will block progression of the disease. We will test an IKKα inhibitor as a potential therapeutic for prostate cancer in this model.

15. SUBJECT TERMS
Prostate cancer, Akt activity, IkappaB kinase, mTOR, animal model

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INTRODUCTION:

Our goal is to understand how IKKα (a key component of the IKK complex that controls NF-κB activitation, see Basseres and Baldwin, 2006) controls Akt in prostate cancer cells, and to determine if IKKα controls development of prostate cancer in an animal model. NF-κB has been reported to be important for prostate cancer progression (Domingo-Domenech et al., 2005). Akt is the key effector in the PI3K pathway and Akt is important in controlling prostate cancer development (Chen et al., 2006). Loss of the tumor suppressor PTEN is sufficient to lead to the development of prostate cancer in an animal model (Wang et al., 2003) and loss of PTEN leads to constitutively active Akt (Hay 2005). The work described here is part of our ongoing effort to understand the interrelationship between the IKK signaling pathway and both mTOR signaling components (mTORC1 and mTORC2) (see Kim et al., 2002; Bjornsti and Houghton, 2004; Dutcher 2004; Dan et al., 2007). Our preliminary data indicated that IKKα associates with the mTORC2 complex in prostate cancer cancers. mTORC2 is the PDK2 activity that phosphorylates Akt at ser473 to control its activity (Sarbassov et al., 2005). Our data show that knockdown of IKKα in prostate cancer cells blocks phosphorylation of Akt at ser473, the known target sequence for mTORC2. Ultimately we want to analyze the role of IKKα in controlling prostate tumorigenesis in an animal model, and to bring

BODY:

Regarding the original Aim 1 goals, we have:

--(Aim 1.1). We have assayed a number of cancer cells to determine whether IKKα controls Akt S473 phosphorylation. In most cancer cells, we find that knockdown of IKKα regulates Akt S473 phosphorylation. **Fig. 1** shows the results of knockdown of IKKα relative to the regulation of Akt phosphorylation in two prostate cancer cells. In these cells, IKKα associates with mTORC2 as shown by co-immunoprecipitation studies (**Fig. 2**). In these studies, immunoprecipitation of IKKα pulled down both Raptor and Rictor, consistent with the association with mTORC1 (Dan et al., 2007) and with mTORC2 (**Fig. 2, left panel**). Immunoprecipitation of Rictor (defining mTORC2) only pulled down mTOR and IKKα and not Raptor (which defines mTORC1) (**Fig. 2, right panel**). In one breast cancer cell we have found that knockdown of IKKα in those cells did not block Akt phosphorylation at ser473. We utilized the PI3K inhibitory LY and found that it did not block association of IKKα with mTORC2 in PC3 cells, indicating that Akt does not drive an association between IKKα and mTORC2 (data not shown).

--(Aim 1.3). We assayed Pdk1 activity following knockdown of IKKα and found that its kinase activity is not diminished (**Fig. 4**). Thus, this data indicates that the effect of IKKα on Akt activity is through mTORC2 and not through Pdk1 (data not shown).

--(Aim 1.4). We have asked whether loss of IKKα will block the upregulation of Akt ser473 phosphorylation found when mTORC1 is inhibited. The upregulation of Akt activity is seen when cancer cells are treated with rapamycin, which blocks mTORC1 and inhibits S6K-phosphorylation. This then blocks the ability of S6K to inhibit IRS-1, a downstream mediator of growth factor-induced Akt activation, allowing growth factor-induced upregulation of Akt (O'Reilly et al., 2006). Our data indicate that loss of IKKα blocks the upregulation of Akt S473 phosphorylation when Raptor (a critical component of mTORC1) is knocked down (**Fig. 3**). This result has important implications for cancer therapy.

Regarding Aim 2 goals, we have:

--(Aim 2.2). We have crossed the IKKα fl/fl animal with the PB-Cre animal. These animals are now being crossed with the PTEN fl/fl animal. Once we have enough founder animals we can begin our studies to determine if IKKα is required for development of prostate cancer downstream of loss of PTEN.

KEY RESEARCH ACCOMPLISHMENTS:

--Clear demonstration that in all prostate cancer cell lines tested (those that are PTEN+ or PTEN-) that IKKα controls Akt activity in these cells.

--Loss of IKKα controls Akt-induced phosphorylation of Foxo3a but not TSC2 (consistent with knockdown of Rictor).

--IKKα is associated with mTORC2 in several cell types, including prostate cancer cells.

--IKKα does not control Pdk1 activity (which controls Thr308 phosphorylation of Akt).

--Loss of IKKα blocks induced Akt activity associated with inhibition of mTORC1, indicating the potential of IKKα inhibition as a mechanism to block both mTORC1 (previous study) and mTORC2, providing a significant advantage of rapamycin treatment.

--Successful cross of IKKα fl/fl animal with PB-Cre animal, in preparation for animal tumor studies.

REPORTABLE OUTCOMES:

--Manuscript is in preparation describing these findings.

--New funding (NIH grant awarded based on these studies)

CONCLUSION:

Our results clearly demonstrate that IKKα is a key regulator of Akt activity in prostate cancer cells (and potentially other cancer cells). Since Akt is a key regulator of oncogenesis in prostate cancer, our work opens the potential that an IKKα inhibitor could be therapeutic in prostate cancer. Our animal model, which is being developed, will provide key genetic evidence for this hypothesis.

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APPENDIX

FIGURE LEGENDS

Figure 1. IKKα positively regulates both Akt and mTOR phosphorylation. (A) PC3 and LNCaP cells were transfected with control siRNA, and siRNAs to IKKα and IKKβ as indicated. The cells were lysed 48 hrs after transfection and the levels of IKKα, IKKβ, and β-tubulin and endogenous phosphorylation of S6K and Akt were determined by immunoblotting with the indicated antibodies. The experiment shown is representative of three different experiments.

Figure 2. IKKα associates with both mTORC1 and mTORC2. (A) and **(B)** Immunoprecipitations of endogenous IKKα (A) or Rictor (B) prepared from PC3 cells were analyzed for endogenous IKKα, mTOR, Rictor or Raptor levels using the indicated antibodies. Mouse (A) or rabbit IgG were used as negative control, and total cell lysate (1% input) indicates expression of mTOR complex.

Figure 3. Loss of IKKα suppresses the loss of feedback control on Akt when mTORC1 is inhibited. PC3 cells were transfected with siRNA IKKα or with siRNA to Raptor. Knockdown of Raptor suppresses mTORC1 activity. The levels of endogenous phosphorylation of S6K, IRS-1, and Akt as well as of IKKα, mTOR and β-tubulin were determined by immunoblotting with the indicated antibodies. Results are representative of three experiments.

Figure 4. **Knockdown of IKKα does not block activation status of PDK1**. Extracts were prepared from PC3 cells knocked down for IKKα which were analyzed with an antibody specific for phospho-S241 on PDK1 (which is reflective of the activity of PDK1).

FIGURES

Figure 1

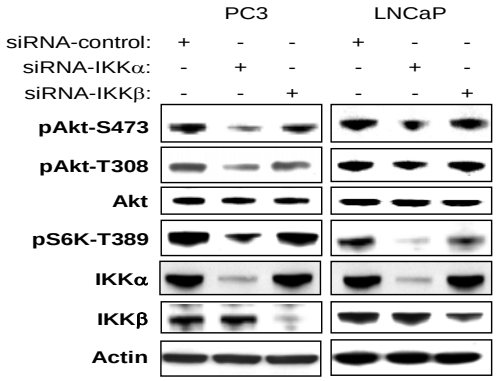

Figure 2

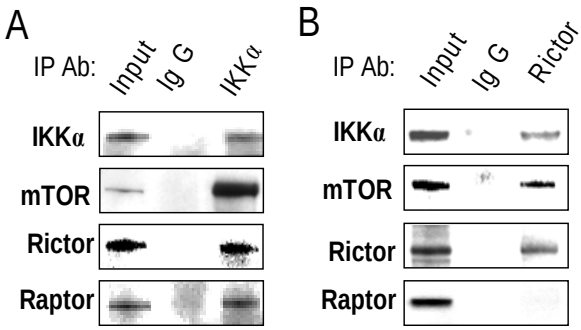
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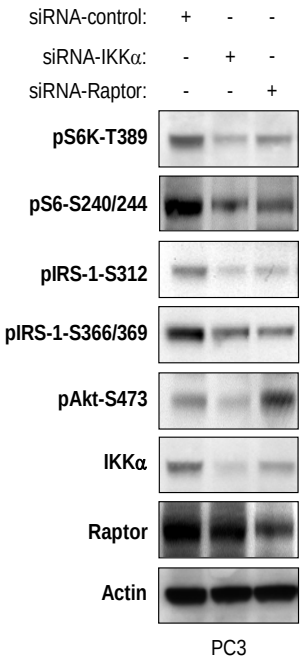
Figure 3


siRNA-control: + - -
siRNA-IKKα: - + -
siRNA-Raptor: - - +
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pS6-S240/244
pIRS-1-S312
pIRS-1-S366/369
pAkt-S473
IKKα
Raptor
Actin
PC3

Figure 4.